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Seroprevalence of Antibodies to *Toxoplasma gondii* in Sheep in Center of Iran

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Abstract: To provide an epidemiologic investigation of the seroprevalence of antibodies to *Toxoplasma gondii* in sheep population in Chaharmahal and Bakhtiari province (Iran) this study was conducted, the province containing five townships, (Shahrekord, Borujen, Farsan, Ardal and Lordegan). One thousands serum samples (600 female and 400 male) were examined to detect antibodies to *T. gondii* by Indirect Fluorescent Antibody Test (IFAT). Serological results showed that the seroprevalence of antibodies to *T. gondii* was 29.1%. The seroprevalence in the townships were 38, 22.5, 32, 35 and 18%, respectively. The results of titration of positive samples showed the following titers: 11% with 1/20, 9.3% with 1/40, 15.1% with 1/80, 28.9% with 1/160 and 35.7% with 1/320 or more. There was no significant difference between the two sexes, while there was a significant difference between the townships located in east and west of the province.

Key words: *Toxoplasma gondii*, antibodies, immunofluorescent, sheep

INTRODUCTION

Toxoplasma gondii, an obligate intracellular protozoan parasite, is a common intestinal coccidian of the cat. Infected cats shed oocysts in feces and contaminating environment (Ettinger, 1983; Jackson, 1989; Soulsby, 1996).

Animals like carnivores and herbivores ingest oocysts and become infected. *T. gondii* encysts in intermediate hosts, cysts can be found in the CNS and muscles of intermediate hosts. Indeed, the zoites of parasite can transfer via placenta and cause abortions and stillbirth in sheep (Levine, 1985; Robert, 1991).

In humans, infection with *T. gondii* may be acquired or congenital, infection is generally acquired from consuming insufficiently cooked meat containing cysts, or by ingesting sporulated oocysts in food, water, or from contaminated environment.

T. gondii infection is one of the most widespread parasitic infections among humans throughout the world. Approximately 30% or more of adult human populations in the world have serum antibodies against *T. gondii* (Dubey and Battic, 1988, Ghorbani, 1981).

Public health concerns associated with *T. gondii* clearly indicate the need for epidemiological investigation of toxoplasmosis. This study was conducted to determine

the seroprevalence of antibodies to *T. gondii* in populations of sheep in Chaharmahal and Bakhtiari in the center of Iran.

MATERIALS AND METHODS

Sample collection: One thousands blood samples were collected from the populations of sheep (Female 600 and Male 400) of five counties of the province (200 for each of them) in the first of summer (2002), by cluster random sampling method.

All the blood samples were collected in serum separator tubes and then centrifuged at 1200x for 10 min. Serum samples were decanted into storage tubes and stored at -30°C until analysis.

Serological examination: All sera from sheep were assayed for antibodies against *T. gondii* by using of Indirect Fluorescent Antibody Test (IFAT). *T. gondii* antigen was prepared by Pasture Institute of Iran, from tachizoite of parasite that killed by formaldehyde 10%. Conjugated serum with Fluorescein Isothiocyanate prepared by Razi Institute of Iran.

After screening the serum samples with dilution of 1:20, for titration of positive serum, dilution of 1:40, 1:80, 1:160, 1:320 were prepared from serums and examined by

the same method. The last titer with positive fluorescent reaction was chosen as titer of serum samples.

A titer more than 1:20 was considered as positive for serum antibodies to *T. gondii* in sheep.

Statistical analyses: A titer more than 1:20 was considered as positive for serum antibodies to *T. gondii*. Seroprevalence was defined as the percentage of positive samples for antibodies to *T. gondii*.

The Chi-Square statistical test (χ^2) was used to determine the difference among different counties and between sexes. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Serum samples from 1000 sheep were collected and checked for antibodies to *T. gondii* by IFAT method. The total population of sheep with serum antibodies to *T. gondii* in the Chaharmahal province was 29.1% (291/1000) (Table 1).

The seroprevalence rate in 5 counties: Shahrekord 38% (76/200), Borujen 22.5% (45/200), Lordegan 18% (36/200), Farsan 32% (64/200) and Ardal 35% (70/200) (Table 1).

The results showed a significant difference ($p < 0.05$), between the counties located in east (Borujen and Lordegan) and west (Shahrekord, Farsan and Ardal) parts of the province, but no significant difference was observed among counties in each of those area.

The number of seropositive sheep did not differ between male (27.8%, 111/400) and female (30%, 180/600), (Table 2).

The titration of positive samples were with the following titers: 11% with 1/20, 9.3% with 1/40, 15.1% with 1/80, 28.9% with 1/160 and 35.7% with 1/320 or more (Table 3).

Table 1: Seroprevalence of antibodies to *T. gondii* in sheep in Chaharmahal and Bakhtiari-Iran

County	No.	Seropositive	Seroprevalence (%)
Shahrekord	200	76	38 ^a
Borujen	200	45	23 ^a
Lordegan	200	36	18 ^a
Farsan	200	64	32 ^b
Ardal	200	70	35 ^b
Total	1000	291	29

a, b, numbers with different superscript in the some column differ significantly, ($p < 0.05$)

Table 2: Seroprevalence of antibodies to *T. gondii* in sheep between two sex in Chaharmahal and Bakhtiari-Iran

Sex	No.	Seropositive	Seroprevalence (%)
Male	400	111	28
Female	600	180	30
Total	1000	291	29

Table 3: Serum titration of seropositive sheep to *T. gondii* in Chaharmahal and Bakhtiari-Iran

Titer	No.	Percent
1/20	32	11
1/40	27	9
1/80	44	15
1/160	84	29
1/320	104	36
Total	291	100

Seroprevalence of antibodies to *T. gondii* in sheep varies depending on the age of sheep, method of serology testing and geographic location. Infection with *T. gondii* is common in sheep, based on reports involving a total of 9654 animals (Fayer, 1981) calculated that the average world wide prevalence of anti Toxoplasma antibodies in sheep was 31% with the range of 0 to 96%.

Blewett and Watson (1983) reported that 33% of sheep in the UK have experienced infection with *Toxoplasma*. Indeed, world wide serologic findings have been shown that, more than 30% of human populations have serum antibodies to *T. gondii*. Several investigators (Claus *et al.*, 1977; Dubey and Beattie, 1988, Dubey *et al.*, 1995; Monica *et al.*, 2002) have determined the seroprevalence of antibodies to *T. gondii* in feline (39-68%) and rodents (0.8-6.3%).

A number of investigators (Dubey *et al.*, 1995; Gamble *et al.*, 1999; Weigel and Dubey, 1995) have shown that farm animals in close contact with cats and rodents have a higher exposure risk to *T. gondii*.

Regarding to the results of this study, a titer of 1:20 was considered as infection of *T. gondii* in sheep, the seroprevalence rate of antibodies to *T. gondii* was 29.1% in the Chaharmahal and Bakhtiari. The lower seroprevalence rate observed among sheep in the east part of the province might be attributed to the climatic properties and the method of husbandry of sheep in these area. In the west and south parts of the province sheep were migrated to the other province (Khozestan) during autumn and winter which substantially had a higher humidity condition and it could be a cause of higher titer to *T. gondii* among sheep in the west and south parts of the province.

Furthermore, the results of this study showed that, there was no significant difference between sexes for antibodies to *T. gondii*. Aristeu *et al.* (2003) in Brazil showed that, the prevalence of antitoxoplasma antibodies in the sheep and goat were 35.3 and 40.4%, respectively. In contrast to the current study a significant difference between sexes in goat and sheep was found.

As the results show, about 89% of positive serum samples showed titer more than 1:20. It may indicate an active infection which can cause not only abortion and congenital problems in pregnant sheep but also in human who are in close contact with infected sheep or consumer who use the milk or meat of affected ones.

The results of our study and other investigators have shown that the prevalence of antibodies to *T. gondii* in sheep is high, for fulfillment the findings, it is necessary to conduct a study for assessment the rate of abortion caused by this parasite in sheep.

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